VACUUM OR MODIFIED ATMOSPHERE PACKAGING AND EDTA-NISIN TREATMENT TO INCREASE POULTRY PRODUCT SHELF LIFE

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Primary Audience: Processing Plant Managers, Regulators, Quality Control Managers and Researchers

SUMMARY

These experiments sought to increase the shelf life of poultry by treatment with a disodium ethylenediametetra-acetate (EDTA) and nisin (NIS) combination and storage under modified atmosphere packaging (MAP) or vacuum packaging (VP). Chicken drummettes were soaked with various combinations of EDTA and NIS for 30 min at 15°C and stored at 4°C. Parts treated with EDTA-NIS stored under VP had significantly lower ($P \leq .01$) total aerobic plate counts than untreated controls stored under aerobic conditions. EDTA-NIS increased shelf life by a minimum of 4 days when packaged in aerobic conditions and a maximum of 9 days when vacuum packed. A second experiment evaluated the VP and EDTA-NIS combinations in more detail. Parts treated with EDTA-NIS stored under VP had significantly different ($P \leq .01$) aerobic counts from parts treated with EDTA-NIS stored under aerobic conditions or untreated control parts. EDTA-NIS treatment increased shelf life 4 to 7 days in the second experiment. The results indicate that a combination of EDTA-NIS treatment and vacuum packaging has the potential to significantly increase the shelf life of raw processed poultry.

Key words: Chelating agents, modified atmosphere packaging, nisin, poultry products, vacuum packaging

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Description of Problem

Spoilage of poultry products is an economic burden to both the producer and the consumer. Developing methods to increase the shelf life of food products is of major research interest to the food processing industry. Several approaches have been investigated, including modified atmosphere packaging (MAP), vacuum packaging (VP), and the use of antimicrobial-chelator systems to inhibit growth of spoilage bacteria on meat products. Various combinations of carbon dioxide, nitrogen, and oxygen have been used in MAP to sustain visual appearance and/or extend the shelf life of meat and meat products. Lactic acid bacteria are the predominant surface microorganisms on meats stored under carbon dioxide. Pseudomonades are responsible for much of the spoilage of meat and meat products and are capable of growing in oxygen/carbon dioxide packaging systems [1]. Concerns have been expressed about the possibility of increasing the numbers of pathogenic anaerobic or micro-aerophilic organisms by using MAP. Davies [2], however, found that the growth of pathogens in MAP was in no instance greater than that in the appropriate controls.

Nisin (NIS), a bacteriocin, is a 3,500-Da polypeptide produced by *Lactococcus lactis* subsp *lactis* that inhibits growth of Gram-positive organisms. Researchers have found that inhibitory NIS activity can be extended to Gram-negative bacteria in the presence of chelators [3]. Stevens *et al.* [4] demonstrated that NIS in combination with EDTA can reduce the population of Gram-negative organisms on broiler carcasses and extend shelf life. EDTA alters the outer membrane structure and increases cell permeability, which allows NIS to penetrate the cell wall. The chelation of magnesium and calcium ions present in the Lipopolysaccharide (LPS) layer of the outer membrane results in LPS destablilization and an increase in cell permeability [5].

The objective of this research was to increase the shelf life of raw poultry products by using an antimicrobial-chelator system in conjunction with modified atmosphere or vacuum packaging. These experiments sought to ascertain the effectiveness of various parameters for atmosphere, NIS levels, and EDTA levels.

Materials and Methods

EXPERIMENT 1

Poultry Products. A commercial processing plant provided 300 post-chill broiler chicken drummettes with skin. The meat was transported on ice to the Food Processing Research and Development Laboratory (FPRDL) at The University of Georgia within 30 min.

EDTA-NIS Treatment. Nine treatments utilizing three concentrations (10, 20, and 50 mM) of EDTA (Sigma Chemical Co., St. Louis, MO) against three concentrations (25, 50, and 100 μg/mL) of NIS (Sigma Chemical Company, St. Louis, MO) were used. Two liters for each EDTA-NIS treatment combination were prepared using sterile distilled water, EDTA, and NIS. The pH of each combination was adjusted to 6.5 with concentrated HCl (Aldrich Chemical Co., Milwaukee, WI). These solutions were stored no longer than 4 hr at room temperature (25°C) before use.

Drummettes were placed in polyethylene containers (30 per container) and covered with the EDTA-NIS solutions and held for 30 min at room temperature. The control group was placed in distilled water. After 30 min, the solution was decanted, and the drummettes were held at room temperature for less than 1 hr until packaged.

Packaging and Storage. Individual drummettes were packaged in Cryovac barrier bags using a Vacuum Packaging Machine (MultiVac, Inc., Kansas City, MO). Three different atmospheres were used: aerobic conditions, vacuum atmosphere, and modified atmosphere (80% O_2 and 20% CO_2). After packaging, the drummettes were stored at 4°C until sampled.

Microbiological Examination. On Days 6, 9, 12, 15, and 18, individual drummettes were removed from storage and weighed, and a volume of sterile saline (0.85%) equal to the weight (weight/volume) was added. The samples were shaken for 1 min and serially diluted (in saline). Total viable cell counts were obtained using Standard Plate Count agar (Difco, Detroit, MI). Dilutions were plated in duplicate, and plates were incubated at 37°C for 48 hr. Counts were obtained using a Protos Plus laser colony counter (Synoptics Ltd., Cambridge, UK). Values were reported as

CFU/mL of rinse. Individual samples were taken until spoilage (CFU $\geq 10^7$) occurred. On Day 21, the 10 groups that had achieved CFU $\leq 10^7$ (as of Day 18) were sampled to depletion of the individually packaged drummettes along with the control group. Values were averaged and reported as mean CFU/mL of rinse.

Statistical Analysis. Data were analyzed using SAS (version 6.11 for Windows), Proc GLM (SAS Institute, Cary, NC). Error term was ($P \leq .01$).

EXPERIMENT 2

Poultry Products. The same procedure was followed as in Experiment 1.

EDTA-NIS Treatment. Seven treatments of 25, 50, and 100 μg/mL NIS, one concentration (50 μg/mL) of EDTA or distilled water were used. Preparation was as in Experiment 1.

Packaging and Storage. Drummettes were packaged (five per Cryovac bag each in individual Stomacher 80 bags) using a MultiVac Vacuum Packaging Machine. Two atmospheres were used, aerobic conditions and vacuum atmosphere. After packaging, the drummettes were stored at 4°C until sampled.

Microbiological Examination. On Days 6, 12, 16, and 19, bags containing five drummettes were removed from storage, each drummette was weighed individually, and a volume of sterile saline (0.85%) equal to the weight was added to each drummette in individual Stomacher bags. Subsequent methodology was as in Experiment 1.

Statistical Analysis. Data were analyzed as in Experiment 1.

Results and Discussion

EXPERIMENT 1

A 3 × 3 × 3 factorial design was conducted using three concentrations of NIS, three concentrations of EDTA, and three atmospheres to evaluate the shelf life of broiler carcass drummettes. Parts treated with EDTA-NIS stored under VP had significantly lower ($P \leq .01$) total aerobic plate counts than untreated controls stored under aerobic conditions. A significant difference ($P \leq .01$) in counts was observed in different storage atmospheres (aerobic condition, VP, or MAP) and at the three levels of EDTA (10, 20, and 50 mM). No difference was observed at the three levels of NIS (25, 50, and 100 μg/mL). The aerobic plate counts at Day 21 for EDTA-NIS-treated parts stored under VP were as much as 2.7 $\log_{10}$ CFU lower than the untreated parts stored under aerobic conditions.

The initial bacterial load of the drummettes was 5×10^2 CFU/mL of rinse. The control group exhibited spoilage at Day 12 of storage as indicated by a population of greater than 10^7 CFU (Table 1). All of the EDTA-NIS combinations packaged under aerobic conditions were spoiled by Day 18.

TABLE 1. Total viable cell counts on EDTA-NIS-treated chicken drummettes at Days 12, 15, and 18 of storage under aerobic conditions at 4°C (Experiment 1)

NIS (μg/mL)	EDTA (mM)	LOG_{10} CFU/mL OF RINSE[A]		
		Day 12	Day 15	Day 18
0	0	7.9	8.7	8.8
25	10	6.5	6.7	8.8
25	20	6.4	7.0	8.4
25	50	5.1	6.6	7.4
50	10	6.2	7.9	8.3
50	20	6.1	6.6	7.7
50	50	5.2	6.4	8.1
100	10	6.7	7.2	8.7
100	20	5.6	7.8	8.3
100	50	5.4	6.9	7.5

[A]Values less than 7.0 $\log_{10}$ CFU/mL indicate spoilage levels have not been reached.

This is a shelf life extension of approximately 4–6 days over the control group (Table 1).

Total viable cell counts for the drummettes stored under VP varied. Five treatment groups (25 μg/mL NIS and 10 mM EDTA; 25 μg/mL and 50 mM EDTA; 50 μg/mL NIS and 10 mM EDTA; 50 μg/mL NIS and 20 mM EDTA; and 100 μg/mL NIS and 10 mM EDTA) exhibited cell counts indicative of spoilage by Day 18 (Table 2). Of the five remaining treatment groups, only two were spoiled on Day 21 (25 μg/mL NIS and 20 mM EDTA; and 100 μg/mL NIS and 20 mM EDTA). The three treatment groups with 50 mM EDTA and various concentrations of NIS were approaching spoilage (CFU$\geq 10^7$) at Day 21 (Table 3). This represents an increase in shelf life of approximately 9 days for these three treatment groups.

The lowest concentration of NIS (25 μg/mL) did not increase the shelf life of the drummettes when stored under MAP regardless of the concentration of EDTA. The higher concentrations of NIS (50 and 100 μg/mL) appeared to extend the shelf life of the drummettes past Day 18 (Table 2). Three treatment groups, 50 μg/mL NIS, 50 mM EDTA, VP; 100 μg/mL, NIS 50 mM EDTA, VP; and 50 μg/mL NIS, 50 mM EDTA, MAP remained below the level of spoilage past Day 21 of storage (Table 3).

EXPERIMENT 2

After evaluating the data obtained in Experiment 1, six combinations of EDTA-NIS were selected for further study under VP. Again, parts treated with EDTA-NIS stored under VP showed a significant difference ($P \leq .01$) in the aerobic counts compared with parts treated with EDTA-NIS stored under aerobic conditions or with the untreated control parts.

The initial bacterial load of the drummettes was 6.3×10^2 CFU/mL of rinse. The untreated control group (Treatment Group 1) was spoiled by Day 12, while none of the other treatment groups (2–7) had

TABLE 2. Total viable cell counts on EDTA-NIS-treated chicken drummettes at Days 12 and 18 of storage under vacuum packaging (VP) or modified atmosphere packaging (MAP) at 4°C (Experiment 1)

NIS (μg/mL)	EDTA (mM)	STORAGE ATMOSPHERE	LOG_{10} CFU/mL RINSE[A]	
			Day 12	Day 18
25	10	VP	6.0	7.8
25	20	VP	4.9	7.0
25	50	VP	8.3	6.6
50	10	VP	6.0	8.4
50	20	VP	5.2	8.4
50	50	VP	3.6	6.2
100	10	VP	5.1	7.3
100	20	VP	4.2	6.8
100	50	VP	4.5	5.4
25	10	MAP	7.2	7.6
25	20	MAP	5.3	7.5
25	50	MAP	5.6	8.3
50	10	MAP	5.9	6.6
50	20	MAP	5.2	6.8
50	50	MAP	4.3	6.5
100	10	MAP	6.3	9.1
100	20	MAP	5.0	6.8
100	50	MAP	5.1	6.5

[A]Values less than 7.0 log_{10} CFU/mL indicate spoilage levels have not been reached.

TABLE 3. Mean total viable cell counts on EDTA-NIS-treated chicken drummettes at Day 21 of storage at 4°C under aerobic conditions, vacuum packaging (VP), or modified atmosphere packaging (MAP) (Experiment 1)

NIS (μg/mL)	EDTA (mM)	STORAGE ATMOSPHERE	LOG_{10} CFU/mL OF RINSE[A]
0	0	Air	9.5
25	20	VP	7.1
25	50	VP	7.0
50	50	VP	6.7
100	20	VP	7.0
100	50	VP	6.7
50	10	MAP	7.8
50	20	MAP	7.4
50	50	MAP	6.8
100	20	MAP	7.6
100	50	MAP	7.1

[A]Values less than 7.0 log_{10} CFU/mL indicate spoilage levels have not been reached.

counts indicating spoilage at that time (Table 4). At Day 16, Treatment Groups 2 and 6 were also spoiled, and a significant difference ($P \leq .01$) was noted when comparing the three spoiled treatment groups (1, 2, and 6) to the other four non-spoiled treatment groups (3, 4, 5, and 7). By Day 19, all of the samples had counts indicating spoilage. In addition to the counts, all samples emitted odors indicating spoilage. At Day 19, significant differences ($P \leq .01$) in counts were observed between Treatment Group 1 and Treatment Groups 3 and 5.

These results are similar to those reported by Shefet *et al.* [6] and Stevens *et al.* [5]. Both research groups were able to extend the shelf life of broiler carcasses and or inhibit Gram-negative organisms by using NIS in combination with chelators (specifically EDTA). Limited information exists concerning the combined effect of antimicrobial-chelator systems and vacuum or modified atmosphere packaging on shelf life of poultry or poultry products.

NIS has broad bactericidal activity against the pseudomonades, lactic acid bacteria, and staphylococci that are found on poultry and poultry products. When combined with a chelator such as EDTA, NIS exhibits bactericidal activity against Gram-negative spoilage organisms as well as several Gram-negative pathogens [3, 4, 5, 6, 7]. This study did not evaluate the effects of these treatments on food-borne pathogens. Previous research on storage under modified atmospheres or vacuum packaging alone has not conclusively yielded increases in the shelf life of poultry products [1].

We used VP and MAP to limit the types of bacteria able to grow on the poultry drummettes. By limiting the growth of certain organisms, we could increase the effectiveness of NIS. The approach yielded an average increase of 6 to 9 days of shelf life in the VP and in the MAP drummettes with EDTA-NIS.

TABLE 4. Mean total viable cell counts on EDTA-NIS-treated chicken drummettes at Days 12, 16, and 19 of storage at 4°C under aerobic conditions or vacuum packaging (VP) (Experiment 2)

TREATMENT	NIS (μg/mL)	EDTA (mM)	n	STORAGE ATMOSPHERE	LOG_{10} CFU/mL OF RINSE[AB]		
					Day 12	Day 16	Day 19
1	0	0	10	Air	7.4^{a}	7.9^{a}	8.5^{a}
2	100	50	10	Air	6.4^{abc}	7.9^{a}	ND[C]
3	0	50	10	VP	5.8^{bc}	6.8^{b}	7.6^{b}
4	25	50	10	VP	5.7^{c}	6.7^{b}	7.8^{ab}
5	50	50	10	VP	6.1^{bc}	6.6^{b}	7.1^{b}
6	100	0	10	VP	6.9^{ab}	8.2^{a}	ND[C]
7	100	50	10	VP	5.5^{c}	6.7^{b}	7.7^{ab}

[A]Values less than 7.0 log CFU/mL indicate spoilage levels have not been reached.

[B]Values with differing lowercase superscripts indicate significant differences ($P \leq .01$).

[C]ND = No data.

CONCLUSIONS AND APPLICATIONS

1. Shelf life of poultry products can be extended by using EDTA, NIS, or EDTA-NIS combinations with either VP or MAP.
2. VP is easier to use and more cost effective because it does not require special atmospheres.
3. A spray application after the chill tank might be the best procedure for applying the EDTA-NIS solution.
4. More work on the method of application is planned.

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